The tadpole of *Physalaemus lisei* Braun & Braun, 1977 (Anura, Leptodactylidae) from southern Brazil

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The tadpole of *Physalaemus lisei* is described from Rio Grande do Sul State, Brazil. Data on the external and internal morphology are presented, along with life history notes. A comparison between the *Physalaemus* species known from Rio Grande do Sul is presented, including all available information from literature.

INTRODUCTION

The genus *Physalaemus* comprises 43 described species, which are distributed in Central and South America, ranging from Mexico to Uruguay and adjacent Argentina (WEBER et al., 2005). Eight species of *Physalaemus* are currently known from the state of Rio Grande do Sul in southern Brazil. *P. biligonigerus*, *P. cuvieri*, *P. gracilis*, *P. henseli*, *P. lisei*, *P. nanus*, *P. riograndensis* and *P.* cf. *gracilis* (KWET, 2001). The external morphology of the tadpoles of five of these species has been previously described: *P. biligonigerus* (FERNÁNDEZ & FERNANDEZ, 1921), *P. cuvieri* (BOKERMANN, 1962; HEYER et al., 1990), *P. gracilis* (LANGONE, 1989), *P. henseli* (BARRIO, 1953, 1964) and *P. riograndensis* (KEHR et al., 2004). No data have yet been published on the larvae of *P. lisei* and *P. nanus*.

Physalaemus lisei Braun & Braun, 1977 occurs in the mountain region of northeastern Rio Grande do Sul (BRAUN & BRAUN, 1977). This species usually inhabits damp woodland and is frequently found in secondary forests or transition zones from woodland to grassland (KWET & DI-BERNARDO, 1999). In this paper, we provide additional data on the life history of *P. lisei* and describe the external morphology of the tadpole for the first time. Our description is compared with descriptions available for congeneric tadpoles occurring in Rio Grande do Sul.

Table 1. – Measurements (in millimetres) of tadpoles of *Physalaemus lisei*. x, mean, s, standard deviation.

Stage	25		27		28		32		34	36	37		40
Sample size	10		2		7		2		1	1	3		1
	x	s	x	s	x	s	x	s			x	s	
Total length	10.9	2.7	13.8	0.2	16.8	1.1	17.5	2.1	17.7	19.1	23.7	0.4	25.2
Body length	4 4	1.4	5 8	0.8	6 7	0.5	7 4	0 2	7.1	7.8	9 8	0 2	9.5
Body width	3.2	1.0	4.2	0.5	4.9	0.4	5.5	0.1	6.0	5.8	7.3	0 6	6.0
Body height	2 6	0 9	3 6	0 5	3 5	0 2	4.5	0 4	4 5	4 9	5 5	0 3	4.5
Tail length	6.5	1.3	8.1	0.5	10.2	0.6	10.1	1.8	10.6	11.2	13.9	0.2	15.7
Eye diameter	0.4	0.1	0.6	0 0	0.7	0.1	0.8	0.1	0 8	0.9	1.1	0.0	1.2
Oral disc width	1 2	0 3	1 3	0 1	1 7	0 1	1.7	0 0	1 8	2 0	2 2	0 0	2.2
Interorbital distance	1 4	0 4	1 5	0 2	1 8	0 1	2.2	0 2	2 3	2.3	3 0	0.1	2.9
Internarial distance	0 7	0 2	0 7	0 1	0 9	0 1	1 0	0 1	1 1	1 2	1 4	0 1	1 4
Eye-nostril distance	0.6	0.2	0.8	0.0	0.9	0.1	1.0	0.1	1.2	1.2	1.7	0.0	1.7
Nostril-snout distance	1 1	0 3	1 2	0 0	1 4	0 1	1.2	0 1	1 4	1 4	2 0	0 1	2.2

Materials and methods

Adult specimens of *Physalaemus lisei* were collected at the Centro de Pesquisa e Conservação da Natureza Pró-Mata, municipality of São Francisco de Paula, Serra Geral region of Rio Grande do Sul, Brazil, at 29°27'-29°35'S and 50°08'-50°15'W (Kwet, 2001). Amplectant pairs were collected at temporary ponds and maintained in captivity until spawning. Foam nests were transferred to an artificial pond measuring 100 × 100 × 40 cm excavated in a field near the collection site. Previously we raised tadpoles in aquaria, but these tadpoles often showed reduced growth rates and malformations of the oral apparatus. To avoid eventual predators, the artificial pond was filled with water two days before the eggs were transferred. The larvae fed on algae and detritus naturally occurring in the pond. We did not provide additional food.

Tadpoles were collected on days 9, 33 and 45 after hatching. Larvae were conserved in 70 % alcohol and deposited in the collection of the MCP (Museu de Ciência e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul, Brazil). We analysed 27 specimens, MCP 3889, nine tadpoles collected on 4 January 1999, MCP 3890, four tadpoles collected on 4 January 1999, MCP 3891, 10 tadpoles collected on 23 December 1998, MCP 3892, 7 tadpoles collected on 23 December 1998, MCP 3895, 10 tadpoles collected on 29 November 1998, MCP 4953, 7 tadpoles collected on 22 January 2001. Measurements were taken to the nearest 0.01 mm with a stereomicroscope (tab. 1), following the terminology of Altig & Mc Diarmid (1999). The labial tooth row formula (LTRF) and developmental stages follow Altig (1970) and Gosner (1960). The internal oral anatomy was studied under a scanning electron microscope. The terminology follows Wassersug (1976).

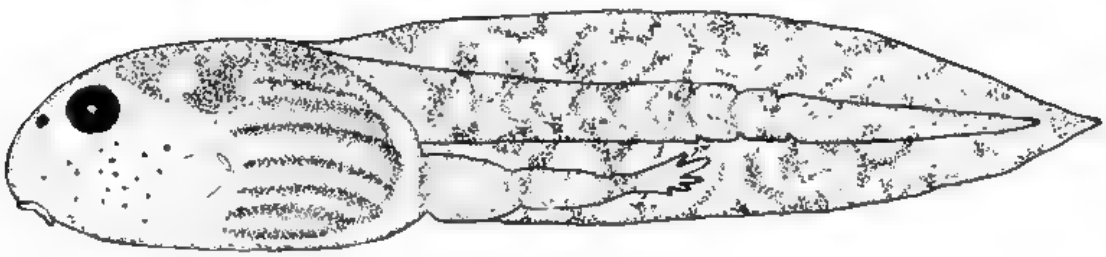

Fig 1 Tadpole of *Physalaemus lisei* lateral view Specimen MCP 3893 (Gosner's stage 37) Scale line 1 cm.

RESULTS

EXTERNAL MORPHOLOGY

Body oval in dorsal view, depressed, approximately 40.1 % (± 2 9 according to the stage) of total length (fig. 1). Snout rounded in dorsal and lateral views. Nostrils round, directed dorsolaterally; closer to eyes than to snout; internarial distance approximately 48 % (± 5.3) of interorbital distance. Eyes dorsal, directed laterally. Spiracle sinistral, located anterior to midbody; lateral wall not free, directed posteriorly. Anal tube dextral, directed posteriorly.

Tail higher than body, about 59 8 % (± 2.9) of total length. Dorsal fin convex, ventral fin almost straight, origin of dorsal fin at body-tail junction. Fins gently tapering to acuminate tip. Caudal muscles not clearly defined

Oral disc emarginated and anteroventral, width 35 4 % (± 6 1) of body width (fig. 2). Lower jaw sheath and upper jaw sheath keratinized. Upper jaw sheath arch-shaped, lower jaw sheath V-shaped, both wider than high and finely serrate. A single row of marginal papillae surrounding oral disk, an extensive rostral gap present, no mental gap. Submarginal papillae absent Labial teeth small, closely spaced. Tooth row formula 2(2)/3(1).

In preserved specimens, gut visible by transparency. Some specimens with a brownish coloration visible on dorsum and on tail muscle. Area surrounding the eyes overall lighter. Tail fins transparent with irregular brownish marks. Lateral line system not visible

INTERNAL ORAL STRUCTURES

Buccal roof (fig. 3A) elongated with semicircular prenarial and postnarial arena Prenarial arena without papillae Ridge present at the middle of the prenarial arena, its width approximately 60 % of the arena's width, with the edge pustulated Postnarial arena with two long lateral ridge papillae. Internal nares oblique in orientation. Narial valve projection ornamented with irregular pustulations. Median ridge slightly inclined towards the rostrum,

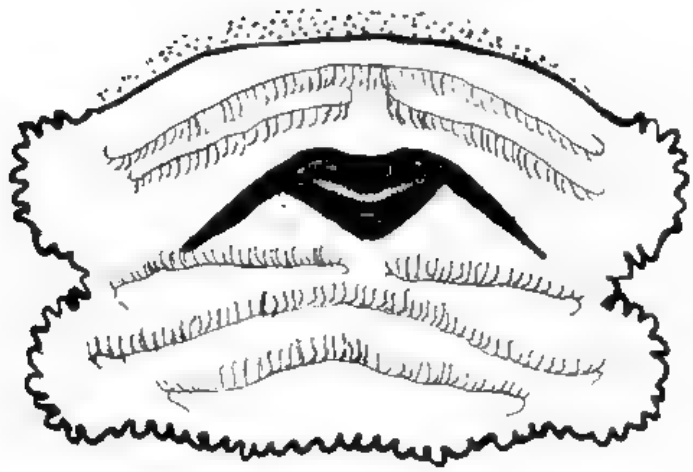

Fig. 2. Tadpole of *Physalaemus lisei* oral disc. Specimen MCP 3893 (Gosner's stage 37). Scale line 2 mm.

overall trapezoidal in shape, its width approximately 50 % of the width of the postnarial arena, and with a pustulated edge. Buccal roof arena U-shaped delimited by four long and finger-like papillae on each side, buccal roof arena with scattered pustulations. Glandular zone with limits in semi-circular form, elevated on lateral parts.

Buccal floor (fig. 3B) triangular, shorter than buccal roof. Presence of six multiple-branching infralabial papillae, pustulated, four near lower beak and two positioned posteriorly. Five lingual papillae localised between the two last infralabial papillae, placed in the medial width of the tongue; four finger-like shaped, two on each side and closely spaced, laterally localised, and a medial bifurcate papilla, larger with ramifications. Buccal floor arena generally with four finger-like papillae on each side and few small pustulations. Ventral velum clearly visible with flaps in the medial part. Pustulations present on the flaps.

Notes on life history

We found 12 foam nests, each of which 3-4 cm in diameter. The minimum number of eggs counted was 397 and the maximum 779, with an average of 539 eggs. Two large, collective foam nests were found at a paddock in the study site. One nest containing 2004 eggs was observed on 20 November 1998 in a small pond measuring 0.75 × 0.75 m, whereas the other containing 1355 eggs was detected on 10 January 1999 in a flooded area.

Larvae of *Physalaemus lisei* were often observed in temporary ponds between or under stones and fallen leaves, scraping algae fixed on stones or particles deposited on the bottom. During feeding, tadpoles move slowly and preferentially use the bottom of shallow water

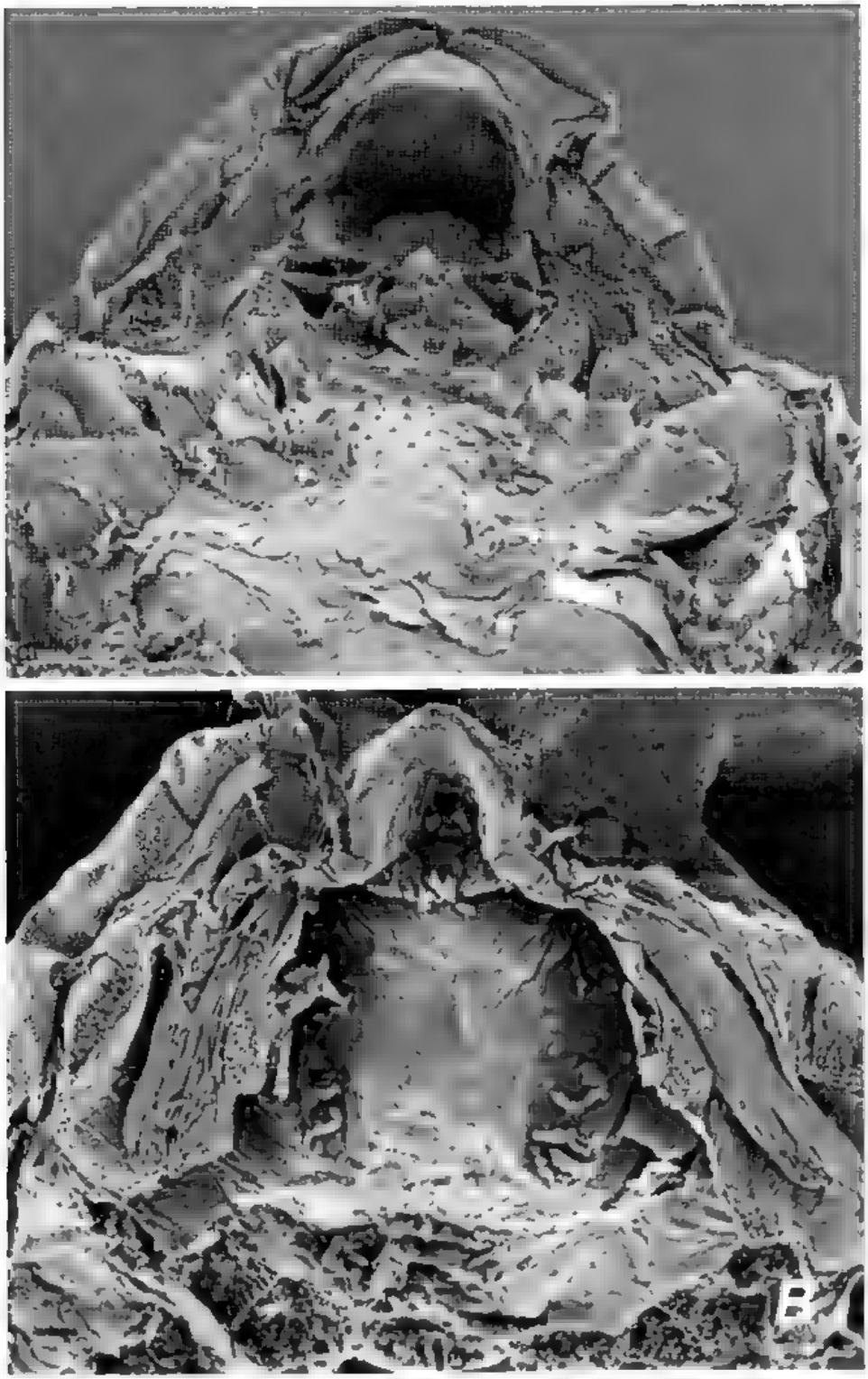

Fig. 3. Tadpole of *Physalaemus lisei*, section of the mouth, Gosner's stage 37. A, buccal roof of oral cavity; scale line: 0.5 mm). B, buccal floor; scale line: 0.2 mm

Discussion

All *Physalaemus* tadpoles from Rio Grande do Sul are similar in their external morphology. In all species, the body is ovoid in dorsal view being wider than high, the vent tube is dextral and the spiracle sinistral. Eyes are dorsolateral, the oral disc is anteroventral and the overall coloration is brownish or greyish. Our measurements taken from larval *P. lisei* varied considerably between different stages, pointing out that morphometric data might not be suitable for the differentiation between tadpoles of different species of *Physalaemus*.

Although variable, the oral morphology allows the differentiation among tadpoles in some species, e.g., tadpoles of species in the *P. cuvieri* species group from Argentina, which can be distinguished solely based on their oral disc morphology (Kehr et al., 2004). Morphological characteristics also allow the distinction betweeen the tadpoles of *P. cuvieri*, *P. henselii*, *P. lisei* and *P. riograndensis* (*P. cuvieri* species group) and of *P. biligonigerus* and *P. gracilis* (*P. biligonigerus* species group) from Rio Grande do Sul. *Physalaemus biligonigerus* and *P. riograndensis* can easily be distinguished from other species by presenting only two lower tooth rows and from each other by their different tooth row formulae: *P. biligonigerus* 2(2)/2 (Fernández & Fernandez, 1921) and *P. riograndensis* 2(2)/2(1) (Langone, 1989) (tab. 2).

In some species of *Physalaemus*, the oral morphology was described using tadpoles from different populations. This led to some confusion. *Physalaemus henselii* was first described by Barrio (1953) with the tooth row formula 2(2)/3(1), whereas the same species was later described as having the formula 2/3(1) (Barrio, 1964). For *P. cuvieri*, Bokermann (1962) and Cei (1980) recorded the formula 2/3(1), but Heyer et al. (1990) reported the formula 2(2)/3(1). For *Physalaemus henselii* and *P. cuvieri*, the formula 2/3(1) was also reported (Bokermann, 1962; Barrio, 1964). These two species can be identified by the number of marginal papillae. Whereas *Physalaemus cuvieri* has a single row of marginal papillae, *P. henselii* has two rows of papillae which are located at the side near of the emargination. These marginal papillae might be also used to differentiate between other species of *Physalaemus*. *Physalaemus cuvieri* and *P. lisei* have a single row of marginal papillae, whereas *P. henselii* and *P. gracilis* present a double row. *Physalaemus cuvieri* can be distinguished from *P. lisei* by having a rostral and a mental gap, whereas *P. lisei* has only a rostral gap. *Physalaemus henselii* and *P. gracilis* can also be differentiated by the presence of gaps: the first has mental and rostral gaps, whereas the second has only a rostral gap.

However, in several species of *Physalaemus* the oral morphology cannot be used for the unambiguous differentiation of tadpoles, e.g., in *P. bokermanni* (Cardoso & Haddad, 1985) and *P. maculiventris* (Bokermann, 1963), which have the same tooth row formula and marginal papillae arrangement as *P. lisei*.

With regard to the foam nest size, *Physalaemus biligonigerus* seems to possess the largest nests within all species of *Physalaemus* known from Rio Grande do Sul, measuring 10-15 cm in diameter (Fernandez & Fernandez, 1921). *Physalaemus cuvieri* has mid sized foam nests of 5-6 cm in diameter containing 300-400 eggs (Kwet & Di-Bernardo, 1999). According to Cei (1980), the diameter is 7-9 cm. *Physalaemus lisei* and *P. henselii* have small sized foam nests, 3-4 cm in diameter. In the foam nests of *P. henselii*, Barrio (1953) found 200-250 eggs and Cei (1980) reported 250-300 eggs. In the present study, we observed egg numbers in *P. lisei*

Table 2 Comparison of oral morphological features of tadpoles of *Physalaemus* species from Rio Grande do Sul (South Brazil)

Species	Reference	Tooth row formula	Marginal papillae row	Rostral gap	Mental gap
P. biligonigerus	FERNANDEZ & FERNANDEZ, 1921	2(2)/2	Single	Present	Absent
P. cuvieri	BOKERMANN, 1962, CEI, 1980	2/3(1)	Single	Present	Present
P. cuvieri	HEYER et al., 1990	2(2)/3(1)	Single	Present	Present
P gracilis	LANGONE, 1989	2(2)/3(1)	Double	Present	Absent
P. henselu	BARRIO, 1953	2(2)/3(1)	Single on the lower labium, double at sides	Present	Present
P henselu	BARRIO, 1964	2/3(1)	Single on the lower labium, double at sides	Present	Present
P lisei	Hoc loco	2(2)/3(1)	Single	Present	Absent
P riograndensis	KEHR et al., 2004	2(2)/2(1)	Single	Present	Absent

varying between 300 and 700 eggs. There are no data available on *P gracilis*. Additional field data are required to distinguish between the foam nests of the different species of *Physalaemus*.

RESUMEN

La larva de *Physalaemus lisei* es descrita por primera vez. Aportamos datos sobre la morfología interna y externa junto con notas sobre la história natural. Comparamos toda la información disponible sobre larvas de *Physalaemus* del estado de Rio Grande do Sul.

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